influenza-pneumonia alone during the 1920 epidemic was about five times as high as the usual rate from influenza and pneumonia in the age periods "0–14" and "45+," and about twenty times as high as the usual rate in the age period 15–44. It appears certain that had it not been for the epidemic of influenza in 1920, the death rates in that year at the ages of 15, 20, and 30 would have shown a decline over the corresponding rates in 1910. How much of a decrease would have been indicated is at the present time difficult to say.

The changes in the death rates have altered materially the complete expectation of life in 1920 as compared with 1910 and 1901. From the figures prepared by the Bureau of the Census and the Metropolitan Life Insurance Co., have been computed the increases or decreases in the expectation of life in 1910 over 1901 and 1920 over 1910, as shown in the following table.

Increase or decrease in complete expectation of life at specified ages: 1910 over 1901 and 1920 over 1910: Original registration States.

Age.	Increase (+) or decrease (−) in—			Age.	Increase (+) or decrease (−) in—		
	1920 over 1901 (years).	1910 over 1901 (years).	1920 over 1910 (years).		1920 over 1901 (years).	1910 over 1901 (years).	1920 over 1910 (years).
Under 1	+5.05	+2.25	+2.80	50	+.28	−.28	+.56
5	+2.21	+1.23	+.98	60	+.13	−.34	+.27
10	+1.92	+1.01	+.91	70	−.17	−.19	+.02
15	+1.85	+.92	+.93	80	+.02	−.05	+.07
20	+1.75	+.74	+1.01	90	+.07	+.08	−.01
30	+1.29	−.19	+1.10	100	+.29	+.27	+.02
40	+.80	−.14	+.94				

The "expectation of life" tells the story in a better way than do the mortality rates. In 1910, at the ages of 40 to 80 the expectation of life had actually decreased over what it was in 1901. But in 1920, in spite of abnormally high mortality, especially in certain age groups, from the influenza epidemic, there was a gain in the expectation of life over 1910 in practically every age shown.

THE RELATIVE PARASITICIDAL VALUE OF ARSPHENAMINE AND NEOARSPHENAMINE.

WITH A DESCRIPTION OF THE TRYPANOCIDAL TEST.

By CARL VOEGTLIN, Professor of Pharmacology, and D. W. MILLER, Scientific Assistant, United States Public Health Service.

About 12 years have now elapsed since the introduction of arsphenamine and neoarsphenamine as remedies for the treatment of syphilis, and yet no conclusive clinical evidence has been furnished as to whether or not these drugs are of equal effectiveness in curing

the disease. The reasons for this lack of information are obvious if the varying nature of the disease in different patients and the length of time required for its treatment are considered. Such factors make the appraisal of the curative value of these drugs very difficult indeed. To be sure, the majority of experienced syphilographers hold the opinion that arsphenamine is more effective than neoarsphenamine, but we have failed to locate any clinical report which would clearly prove this point.

The great difficulties encountered in the solution of this important question in the clinic have forced various investigators to resort to experimental procedures in order to settle it. The great advantage of the latter method consists in the fact that experimental infections are subject to accurate control. Ehrlich and Hata (1911) conducted comparative therapeutic tests with chicken spirillosis and relapsing fever in mice, and arrived at the conclusion that 0.6 gram of arsphenamine is equivalent in curative value to 0.9 gram of neoarsphenamine, a ratio of 1 to 1.5. Castelli (1912) reports a ratio 1 to 1.78 for relapsing fever in mice, 1 to 1.7 for chicken spirillosis, and 1 to 1.5 for rabbit syphilis. Schamberg, Kolmer, and Raiziss (1920), established on rats infected with *Trypanosoma equiperdum* an average ratio of 1 to 1.75, in testing out various lots of arsphenamine and neoarsphenamine. Voegtlin and Smith (1921), also studied this question on rats infected with *Trypanosoma equiperdum*, using a technique which permits accurate measurement of the killing power of the drugs for a definite number of parasites in a given time. The average ratio found was 1 to 1.7.

In view of the great practical importance of this question in the control and eradication of syphilis, it appeared desirable to secure further data.

THE TRYPANOCIDAL TEST.

In the selection of any method for testing the relative parasiticidal value of such drugs as arsphenamine and neoarsphenamine, several considerations have to be taken into account. Accuracy above all is a prime requisite, and this should not be sacrificed for the sake of saving either labor or expense. At first thought it would appear that for the study of the curative value of these two drugs rabbits infected with *Treponema pallidum* should be chosen. However, this infection shows considerable variations in different rabbits and sometimes tends to recover spontaneously, thus making the results not quite reliable from the quantitative standpoint. It goes also without saying that it is utterly impossible in work on rabbit syphilis to gain an idea of the absolute number of parasites present in the animal at the time of the injection of the drug. It is therefore impossible to determine the absolute amount of drug necessary to kill a definite

number of parasites within the host. Our extensive experience with rats infected with *Trypanosoma equiperdum* has convinced us, and we fully agree in this matter with Schamberg, Kolmer, and Raiziss (1920) that this disease lends itself admirably for our purpose.

Nature of infection.—*Trypanosoma equiperdum* is the parasite which is responsible for so-called horse syphilis. This organism produces in horses and rabbits a chronic disease characterized by marked tissue changes. In albino rats the infection is characterized by its rapid progress, leading to the appearance of parasites in the blood and their rapid multiplication therein, so that the animal dies regularly within a few days. The natural resistance of rats to this organism is almost nil, for recent experiments have shown us that one or very few parasites injected intravenously will always produce death within 10 days. The multiplication of the parasites in the blood proceeds in logarithmic proportion, approximately 7 hours being required to double the number of parasites in the blood stream. The virulence of this parasite is remarkably constant once it has been carried in rats for a week or two. The principle of the trypanocidal test is based on the fact that a well-defined dose of the drug is required to kill a certain number of parasites within the blood of infected rats, i. e., the parasiticidal power of the drug is measured in terms of the number of parasites killed.

Technique of test.—Healthy albino rats from one breeding strain and weighing about 50 to 60 grams are put on a diet of milk, bread, and oats until they reach a weight of 100 to 150 grams, when they are ready to be used for the test. The animals should be nonpregnant. A series of such rats are inoculated with citrated blood from a seed rat. The latter, showing about 200,000 trypanosomes per cubic millimeter is bled by decapitation directly into 5 c. c. of saline solution containing 2 per cent sodium citrate. About 0.5 c. c. of this suspension of parasites is injected intraperitoneally into each rat. The amount of citrate used can be varied according to the number of parasites present in the seed rat. The above amount of the suspension will usually produce in 24 hours an infection of about 100,000 parasites per cubic millimeter of blood, and the animals will die if left untreated, as a rule two days later.

Counting the parasites.—The method of counting the parasites in the blood of an infected animal is essentially the one proposed by Kolmer (1915), who made use of the ordinary blood pipettes for counting red cells. The tip of the tail is nipped off. Slight pressure beginning at the base of the tail and moving up to the tip is sufficient to produce free bleeding. The first drop of blood appearing should be discarded. The blood pipette is then filled to the 5 mark with blood and made up to the 10 mark with diluting fluid. The pipette should be shaken for three minutes in order to obtain a homogeneous

distribution of the parasites. The suspension is then placed in a counting chamber and allowed to settle for five minutes. As the red cells are laked by the diluting fluid, the parasites are easily distinguished from the leucocytes. The number of squares to be counted will, of course, depend somewhat upon the number of parasites present. With a large number, fewer squares have to be counted.

The diluting fluid.—The diluting fluid is prepared as follows: 20 milligrams of crystal violet is dissolved in 200 c. c. of water, with slight warming; 700 milligrams of NaCl is added and 2 c. c. of formaldehyde, and the solution is cooled and filtered. This fluid should be made up fresh every day.

Injection of the drug.—The injection of the drug to be tested is carried out as follows: The drug is dissolved according to the usual procedure in distilled water, avoiding all unnecessary shaking and adjusting the concentration in such a way that the volume of the desired dose is within from 0.3 to 0.9 c. c. An accurately calibrated tuberculin syringe, provided with a 26-gauge Luer needle, is then filled with the freshly prepared solution, and the drug is immediately injected at a slow rate into the leg vein previously exposed by skin incision. It is not necessary to use anesthesia for this very simple operation. The rat is tied to a board provided with four nails to which the four legs are fastened by means of strings. The number of trypanosomes per cubic millimeter of blood should be within 100,000 to 250,000, preferably 100,000 to 150,000.[1] The choice of a uniform grade of infection is very important for accurate work, as Voegtlin and Smith (1920) have shown and subsequent experience has confirmed.

The minimum effective dose.—In order to compare the trypanocidal efficiency of various lots of arsphenamine or neoarsphenamine, graded doses of the drug are injected into a series of animals. It has been found that a variation of approximately 50 per cent between successive doses (1, 1.5, 2.25, 3.75, 5, 7.5, 10, etc.) is all that can be expected of the accuracy of the test. This is due to the fact that quantitative differences in the metabolism of the drug and the rate of excretion of the arsenic in different rats are sufficiently great to produce considerable variations in parasiticidal action. These variations make it necessary to test at least five rats at each dose. At the end of 24 hours after the injection of the drug, the number of parasites in the tail blood is again determined, first by a preliminary examination of a drop for the presence or absence of parasites, and then by a count of the blood specimens which were found positive in the

[1] If the parasite count should be below 100,000, treatment is delayed until later in the day, when the required stage of the disease has been reached. Thus, it sometimes happens that in a series of rats inoculated with the same volume of a given suspension, the number of parasites in the morning of the day instead of being 100,000 is 50,000. In this case the animals can be used in the afternoon of the same day.

preliminary examination. The method permits an accurate count of 1,000 or more parasites per cubic millimeter. Below 1,000 the count is unreliable, and therefore we have refrained from making counts in such cases and merely go by the result of the drop examination, calling it a trace, when only one parasite is found in several different microscopic fields of a fairly thick preparation.

The minimum effective dose is the dose required to bring the parasitic count within 24 hours to a trace or negative, or, in other words, the dose which kills from 100,000 to 250,000 parasites per cubic millimeter of blood. As a rule the blood is again examined at the end of 48 and 72 hours after treatment.

Extensive experience has shown us that with arsphenamine and neorarsphenamine the maximum effect upon the parasites is exerted within the first 24 hours, though occasionally a count of 1,000 or less may become trace or negative in 48 or 72 hours.

It should be stated for the benefit of those who have not had any experience with work of this nature that particular caution should be exercised not to use rats which have received at any previous time an injection of an arsenical, as this may lead to the production of arsenic resistant parasites and may thus render the result erroneous.

Value of trypanocidal test.—Our experience with this test during the last four years in the study of a large number of arsenicals has convinced us of its accuracy and practical importance. The objection which might be raised—namely, that the test really does not establish the relative parasiticidal efficiency of arsphenamine and neoarsphenamine with regard to *Treponema pallidum*—is not justified, for the reason that the curative ratio of these two drugs as established by Ehrlich and Hata and Castelli in spirochete infections, including rabbit syphilis, is practically the same as that determined by Voegtlin and Smith (1921) by means of this test. Every arsenical which was shown to be efficient in the treatment of rabbit and human syphilis was also shown to possess a high efficiency by means of the trypanocidal test, and vice versa. Reference is made only to cacodylic acid, monomethylarsenic acid (arrhenal), and ethylarsenic acid (monarson), substances which failed to free the blood of injected rats from parasites except with doses appraoching the lethal dose. These negative observations were later on confirmed by Nichols on rabbit syphilis.

The strain of *Trypanosoma equiperdum* used in our work has not shown any change in resistance to a given drug during the last four years, the minimum effective dose remaining the same as long as the strain is carried in rats. If, for the sake of economy, it is necessary to preserve the strain in rabbits, we advise that before the strain is again used in the rat it be carried for at least two weeks in the rat

before being used for testing out drugs in a quantitative, comparative way. The use of a standard preparation of arsphenamine (or 3 amino 4 hydroxyphenylarsenious oxide) to test the absolute drug resistance of the strain of trypanosomes is strongly recommended. By the use of such a standard we have shown that the drug resistance of our original strain has not changed during four years, and with thousands of transfers in rats, truly a remarkable example of persistence of an inherited biological property.

ARSPHENAMINE AND NEOARSPHENAMINE.

The above-described test was applied to 13 lots of arsphenamine and 15 lots of neoarsphenamine of different manufacture. Most of these preparations, with exception of neoarsphenamine Brand B, Lot 1, and Brand E, Lot 1, were of recent manufacture (last quarter of 1921). The doses of the drugs are expressed either as milligrams or as number of c. c. of a 1/100 arsenic equivalent solution per kilo body weight. The former mode of expression uses the absolute weight of the drug irrespective of its arsenic content, the latter permits a direct comparison of the parasiticidal value of the arsenic in the two drugs and is the preferable expression for purely scientific purposes. A 1/100 arsenic equivalent solution would be a 1/200 molecular solution of arsphenamine or neoarsphenamine if the impurities, such as inorganic salts, etc., are ignored. In addition to the parasiticidal power of the preparations, their toxicity was also established by the official toxicity test.

It will be seen from an examination of the accompanying tables that the trypanocidal power of arsphenamine of different manufacture is remarkably constant, varying only between 3 and 4.5 c. c. 1/100 arsenic equivalent solution per kilo body weight. This is quite in contrast with the great variability encountered with neoarsphenamine, which ranges from 2.25 c. c. for the most efficient preparation, to 7.5 for the least effective one. As far as the toxicity is concerned, both arsphenamine and neoarsphenamine show considerable variation, and particular attention is called to the low toxicity of some of the drugs studied as compared with commercial preparations produced about two years ago. Three lots of Brand B arsphenamine show a lethal dose of 280 mg., and a tolerated dose of 260 mg. per kilo, whereas the official requirements call for 120 mg. Again, in the case of neoarsphenamine, all of the lots examined passed at 300 mg. per kilo with the official test, the lethal dose varying between 360 and 520 mg. per kilo. This plainly indicates that the average arsphenamine and neoarsphenamine produced at the present time is considerably less toxic than were similar preparations manufactured two years ago, and this means, of course, that the process of manufacture has been considerably improved. This decrease in toxicity appears to have been accompanied by a corresponding decrease in

parasiticidal power, as the minimal effective dose two years ago used to be on an average 3 c. c. or less for arsphenamine and 3 c. c. for neoarsphenamine. This is further seen by a comparison of the therapeutic ratio $\frac{\text{m. l. d.}}{\text{m. e. d.}}$ of the present lots and those studied earlier. The average ratio for arsphenamine is 20 and it was 17 two years ago; for neoarsphenamine it is 24 and two years ago it was 28. Evidently there is very little change in the ratio as would be expected if the toxicity and parasiticidal power of these drugs are somewhat related.

The most important point brought out by this investigation is the fact that *neoarsphenamine is a much more variable product than arsphenamine as far as parasiticidal power is concerned*, and this confirms the conclusions drawn by Voegtlin and Smith (1921) from a previous study. The physician using neoarsphenamine can therefore never be sure of obtaining a product of constant potency, unless he uses continuously one and the same lot; whereas the therapeutic potency of arsephenamine of different manufacture appears to be much more constant. In this respect, therefore, arsephenamine is decidedly superior to neoarsphenamine. It is conceded, however, that the technique for the clinical administration of the latter is simpler, and on this account neoarsphenamine appeals to the physician more than arsephenamine.

After this manuscript had been completed, an important report by Dale and White, of the National Institute for Medical Research in London, came to our attention. In this report it is shown that the trypanocidal test (modified slightly by the authors [1]) is "a very valuable index, if not an accurately quantitative measure of the therapeutic activity of different samples of neoarsphenamine on syphilis in man." The results obtained with six different lots of neoarsphenamine on mice infected with *Trypansoma equiperdum* and on cases of primary syphilis are given in the following table:

Preparation.	Minimal curative dose for mice infected with *T. equiperdum*.	Proportion of human cases in which spirochetes were detected 18–20 hours after injection of 0.45 g.
B3	0.015	0 out of 6.
A2	.02	0 out of 4.
A3	.02	1 out of 6.
C3	.02	1 out of 6.
C2	.03	3 out of 6.
B2	.05	9 out of 10.

These data therefore complete the evidence required to prove the practical value of the trypanocidal test.

[1] Mice were used instead of rats. With mice it was necessary to examine the blood for three days after the injection to obtain the maximum parasiticidal effect. Various workers in this laboratory have found work with rats considerably less difficult than work with mice. The choice of the host, whether rat or mouse, is to be decided by the person who uses the trypanocidal test.

CONCLUSIONS.

1. The results obtained in this investigation confirm previous data from this laboratory to the effect that arsphenamine of different manufacture is fairly uniform in parasiticidal power, whereas neoarsphenamine shows great variations.

2. The toxicity of the average commercial arsphenamine and neoarsphenamine manufactured at the present time is considerably lower than that of preparations found on the market two years ago.

3. The technique of the trypanocidal test as elaborated in this laboratory during the last few years is described in detail.

REFERENCES.

Castelli, G.: Ztschr. f. Chemother., orig., 1912–13, p. 321.

Dale, H. H., and White, C. F.: The Lancet, Apr. 1922, CCII, p. 779.

Ehrlich, Paul, and Hata: Die experimentelle Chemotherapie der Spirillosen, Rebman, 1911.

Kolmer, J. A.: Jour. Inf. Dis., 1915, XVII, p. 79.

Nichols, H. J.: Jour. Am. Med. Assoc., 1921, LXXVI, p. 1335.

Schamberg, Kolmer, and Raiziss: Am. Jour. Med. Sciences, 1920, CLX, p. 25.

Voegtlin, Carl, and Smith, Homer W.: Jour. Pharmacol. & Exp. Ther., 1920, XV, 453.

Voegtlin, Carl, and Smith, Homer W.: Jour. Pharmacol. & Exp. Ther., 1921, XVI, p. 449.

TABLE I.—*Arsphenamine—Trypanocidal power of various lots of different manufacture: Trypanosome counts 24 hours after injection with drug.*

(The initial counts in practically all cases ranged from 100,000 to 250,000.)

Dose: 1/100 arsenic equiv. solution per kilo.—	Brand A.			Brand B.			Brand C.			Brand D.		Brand E.	
	Lot 1.	Lot 2.	Lot 3.	Lot 1.	Lot 2.	Lot 3.	Lot 1.	Lot 2.	Lot 3.	Lot 1.	Lot 2.	Lot 1.	Lot 2.
c. c.													
2	22,000	8,000	11,000	3,000	15,000	19,000	64,000	12,000	18,000	16,000	10,000	8,000	34,000
	5,000	7,000	7,500	2,000	7,000	Trace.	61,000	8,000	13,000	10,000	8,000	8,000	19,000
	3,500	4,000	2,000	Trace.	6,000	Trace.	38,000	6,000	7,500	6,000	6,000	5,000	10,000
	3,000	Trace.	Trace.	Trace.	1,000	Trace.	35,000	4,500	6,000	5,000	3,000	4,500	4,000
	Trace.	—	—	—	—	—	14,000	—	4,000	Trace.	Trace.	—	1,000
			—										
3	Trace.	4,000	—	2,000	10,000	6,000	22,000	3,000	2,000	Trace.	16,000	1,000	9,000
	Trace.	Trace.	—	2,000	—	Trace.	9,000	Trace.	Trace.	Trace.	2,000	Trace.	6,000
	Trace.	Trace.	—	1,000	—	Trace.	Trace.	—	—	Trace.	—	Trace.	5,000
	—	—	—	Trace.	—	—	Trace.	—	—	—	—	Trace.	—
	—	—	—	Trace.	—	—	—	—	—	—	—	—	—
	—	—	—	—					—				
4.5	—	—	—	—	—	—	Trace.	Trace.	Trace.	—	—	—	Trace.
	—	—	—	—	—	—	Trace.	Trace.	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE II.—*Arsphenamine—Trypanocidal power of various lots of different manufacture: Trypanosome counts 48 hours after injection with drug.*

Dose: 1/100 arsenic equiv. solution per kilo.—	Brand A.			Brand B.			Brand C.			Brand D.		Brand E.	
	Lot 1.	Lot 2.	Lot 3.	Lot 1.	Lot 2.	Lot 3.	Lot 1.	Lot 2.	Lot 3.	Lot 1.	Lot 2.	Lot 1.	Lot 2.
c. c.													
2	++++ ++++ +++ +++ ++	+++ + + + Trace.	+++ ++ + + —	+ + Trace. Trace. —	++ ++ + + Trace.	++ + + Trace. —	+++ +++ +++ ++ —	++ ++ + + —	++ ++ ++ + +	+++ +++ ++ + +	+ + + + +	++ + + + —	++ ++ ++ + +
3	+ — — — —	+++ — — — —	— — — — —	+ + + + —	++ Trace. — — —	++ + Trace. — —	++ ++ + — —	+ + — — —	+ — — — —	+ + Trace. — —	+ + — — —	Trace. — — — —	+ ++ + — —
4.5	— — — — —	— — — — —	— — — — —	— — — — —	Trace. — — — —	Trace. Trace. — — —	Trace. Trace. — — —	+ — — — —	+ — — — —	— — — — —	Trace. — — — —	— — — — —	Trace. — — — —

TABLE III.—*Arsphenamine—Toxicity of various lots of different manufacture.*

S=Animal survived 48 hours following the injection of the drug.
D=Animal died within 48 hours after the injection of the drug.

Dose, mgm. per kilo.	Brand A.			Brand B.			Brand C.			Brand D.		Brand E.	
	Lot 1.	Lot 2.	Lot 3.	Lot 1.	Lot 2.	Lot 3.	Lot 1.	Lot 2.	Lot 3.	Lot 1.	Lot 2.	Lot 1.	Lot 2.
120	S S S S S	S S	S S S S S									S	S S S S S
140	S S S S S	S S S S S	S S D D D	S S	S S	S S		D		S S S S S	S S S S D	S S S S S	S D D D
160	S S S S S	S S S D D	S D D D D	S S	S S	S S	S S	S	S	S S S S S	S S D D D	S S	D D D D
180	S D D D D	S S S S S	D	S S	S S	S S	S S S S S	S	S S S S S	S D D D D D	S D D D D	S S	D D D D
200	D	S D D D D		S S	S S	S S	S S S S S	S S S S S	S D D D D	D D D D D	S D D D D	S S	D D D D
220				S S S S S	S	S	S S S S S	S S S S S	S D D D D		D		D
240				S S S S S	S	S	S D D D D	D D D D D	D D D D				
260				S S S S S	S S S D D	S S S D D	D D D D D	D D D D D					
280				S S D D D	S S D D D	S D D D D							

TABLE IV.—*Neoarsphenamine—Trypanocidal power of various lots of different manufacture: Trypanosome counts 24 hours after injection with drug.*

[The initial counts in practically all cases ranged from 100,000 to 250,000.]

Dose: 1/100 arsenic equivalent solution per kilo—	Brand A.				Brand B.				Brand C.				Brand D.		Brand E.
	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 1.	Lot 2.	Lot 1.
c. c.															
1.5	218,000	396,000	66,000	18,000	61,000	29,000	50,000	210,000	720,000	1,288,000	.....	600,000	15,000	210,000	.....
	210,000	172,000	52,000	10,000	25,000	27,000	39,000	122,000	640,000	1,224,000	.....	520,000	12,000	98,000	.....
	100,000	8,000	37,000	9,000	12,000	18,000	28,000	49,000	35,000	36,000	.....	46,000	4,500	40,000	.....
	44,000	8,000	11,000	6,500	.....	.....	.....	.....	25,000	32,000	.....	45,000	Trace.	16,000	.....
	17,000	Trace.	7,000	4,000	.....	.....	.....	.....	19,000	—	.....	26,000	—	15,000	.....
	—	.....	Trace.	.....	.....	.....	.....	.....	.....	.....	.....	.....	—	.....	.....
	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	—	.....	.....
2.25	Trace.	14,000	12,000	132,000	440,000	1,400,000	720,000	51,000	28,000	608,000	376,000	548,000	81,000	39,000	1,152,000
	Trace.	10,000	5,000	29,000	336,000	92,000	116,000	49,000	15,000	39,000	262,000	396,000	41,000	16,000	700,000
	—	9,000	3,500	10,000	240,000	40,000	27,000	37,000	14,500	22,000	165,000	52,000	31,000	15,000	640,000
	—	Trace.	3,000	8,000	33,000	37,000	23,000	20,000	14,000	18,000	47,000	37,000	21,000	9,000	520,000
	—	—	Trace.	8,000	31,000	33,000	8,000	8,000	6,500	—	21,000	17,000	7,000	Trace.	480,000
	—	—	—	Trace.	9,000	.....	.....	.....	6,000	.....	13,000	.....	5,000	—	.....
	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Trace.	.....	.....
3.5	—	Trace.	—	—	9,000	20,000	22,000	21,000	—	22,000	84,000	36,000	19,000	16,000	720,000
	—	—	—	—	Trace.	15,000	6,000	9,000	—	17,000	18,000	34,000	16,000	9,000	680,000
	—	—	—	—	Trace.	2,500	6,000	6,000	—	14,000	17,000	10,000	3,000	Trace.	464,000
	.....	—	—	—	—	Trace.	4,500	5,000	—	1,000	8,000	Trace.	2,000	Trace.	27,000
	.....	—	—	—	—	—	Trace.	5,000	—	Trace.	3,000	Trace.	Trace.	Trace.	12,000
	.....	—	—	—	—	.....	—	Trace.	—	—	Trace.	.....	Trace.	Trace.	11,000
	.....	.....	.....	.....	.....	.....	.....	.....	—	.....	.....	.....	Trace.	.....	.....
	.....	.....	.....	.....	.....	.....	.....	.....	—	.....	.....	.....	.....	.....	.....
	.....	.....	.....	.....	.....	.....	.....	.....	—	.....	.....	.....	.....	.....	.....
5.0	—	—	—	—	—	1,000	1,500	Trace.	25,000	Trace.	12,000	15,000	Trace.	Trace.	133,000
	—	—	—	—	—	Trace.	Trace.	—	Trace.	—	4,000	7,000	Trace.	—	126,000
	—	—	—	—	—	Trace.	Trace.	—	—	—	—	4,000	Trace.	—	Trace.
	.....	.....	.....	—	—	—	—	—	—	—	—	Trace.	—	—	Trace.
	.....	.....	.....	—	.....	—	—	—	—	—	—	Trace.	—	—	—
	.....	.....	.....	—	.....	.....	—	—	—	—	.....	—	.....	.....	—
	.....	.....	.....	.....	.....	.....	.....	—	—	.....	.....	.....	.....	.....	.....
	.....	.....	.....	.....	.....	.....	.....	.....	—	.....	.....	.....	.....	.....	.....
	.....	.....	.....	.....	.....	.....	.....	.....	—	.....	.....	.....	.....	.....	.....
7.5	.....	.....	.....	.....	.....	Trace.	Trace.	—	—	Trace.	—	Trace.	—	—	Trace.
	.....	.....	.....	.....	.....	Trace.	—	—	—	—	—	—	—	—	Trace.
	.....	.....	.....	.....	.....	—	—	.....	—	—	—	—	—	—	—
	.....	.....	.....	.....	.....	—	—	.....	.....	.....	Trace.	—	—	—	—
	.....	.....	.....	.....	.....	—	—	.....	.....	.....	.....	—	—	—	—
	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	—	.....	.....	.....

TABLE V.—*Neoarsphenamine—Trypanocidal power of various lots of different manufacture: Trypanosome counts 48 hours after injection with drug.*

Dose: 1/100 arsenic equivalent solution per kilo—	Brand A.				Brand B.				Brand C.				Brand D.		Brand E.
	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 1.	Lot 2.	Lot 1.
c. c.															
1.5	++++ ++++ ++++ ++++ ++ −	+++++++ ++++++ + + −	+++ +++ +++ ++++ − −	++ ++ + + +	++++ +++ +++	+++ +++ ++++	++++ ++++ ++++	++++ +++ +++	+++++ +++++ +++ +++ +++	++++ ++++ ++++ ++++ −	....	++++ ++++ +++ +++ ++	++++ ++++ ++ + − −	+++ +++ +++	....
2.25	+ + − − − −	+++ ++ + − − −	+++ +++ ++ Trace. − −	+++ ++ ++ + + −	+++ +++ +++ +++ +++ ++	++++ ++++ ++++ ++++ ++++	++++ ++++ ++++ ++++ ++++	+++ +++ +++ +++ +++	+++ + + + + +	+++++ +++ +++ +++ −	++++ ++++ ++++ +++ ++ ++	++++ ++++ +++ +++ +++	++++ +++ ++++ +++ +++ +++ +++	++++ +++ +++ +++ +++ +	++++ ++++ ++++ +++ +++ ++++ .....
3.5	− − −	+ − − − − −	− − − − − −	− − − − − −	+ + − − − −	++ ++ ++ ++ − −	+++ ++ ++ Trace. Trace. −	++ + + + Trace. Trace.	− − − − − − − −	+++ ++ ++ + Trace. −	+++ +++ ++ ++ + + ...	+++ ++ ++ Trace. −	+++ +++ + + − + −	+++ +++ + − − −	++++ +++ + − − − ....
5.0	− − −	− − −	− − −	− − − − − −	− − − −	+ + − − −	Trace. Trace. Trace. − − −	Trace. − − − − −	++ Trace. − − − − − −	+ − − − − −	++ ++ − − − −	++ ++ ++ + + −	+ + + + −	+ − − − −	+++ +++ − − − − .....
7.5	....	....	....	....	....	− − − − −	− − − − −	− − −	− − −	Trace. − − − − −	− − − − − − − −	− − − − − − − −	− − − − −	− − − − −	− − − − − ....

TABLE VI.—*Neoarsphenamine—Toxicity of various lots of different manufacture.*

S = Animal survived 8 days following the injection of the drug.
D = Animal died within 8 days after the injection of the drug.

Dose, mgm. per kilo.	Brand A.				Brand B.				Brand C.				Brand D.	
	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 1.	Lot 2.
240	S S S S S	....	....	S S S S D	S S S	....	S S S	S S S	....	....	....	....	....	....
280	S S S S S D S D	S S	....	S S S S S D	..... S S	S S	....	....	....	....	....	....	....	....
300	S S S S S	S D S D S D S D S D	S S S S S D	....	....	....	....	....	S S S S S	S S S S S	....	S S S S S	S S S S S D	S S S S S
320	....	....	....	D D D D D	S S S	S S D	S S S	S S S	....	....	....	....	....	....
340	....	S D S D S D S D S D	....	....	....	....	....	....	....	....	....	....	....	....
360	S S S S S S D S D	....	S S S S S S	....	S S S S S	S S S D D	S S S S S	S S S S S	S S S D	S S S S S	....	S S S S D	D D D D D	D D D D D
380	S S S D D	....	....	....	....	....	....	....	....	....	....	....	....	....
400	S S S S S S D S D	D D D D D	....	....	S S S S S	S S S S S	S S S S S D	S S S S S D	S D D D	....	....	....	D D D D D	....
420	....	....	D D D D D	....	....	....	....	....	....	S S S S S	S S D D D	....	....	....
440	S S D S D	....	....	....	S S S S S	S S S D D D	S D D D D	D D D D D	....	....	....	S S S D D	....	....
460	....	....	....	....	....	....	....	....	....	....	S S S S S	....	....	....
480	S S D D	....	....	....	S S S S D	D D D D D	S S S S S D	....	....	S S S D D	....	D D D D D	....	....

TABLE VI.—*Neoarsphenamine—Toxicity of various lots of different manufacture*—Continued.

S=Animal survived 8 days following the injection of the drug.
D=Animal died within 8 days after the injection of the drug.

Dose, mgm. per kilo.	Brand A				Brand B.				Brand C.				Brand D.	
	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 1.	Lot 2.
520	.....	.....	.....	.....	S D	S	.....	.....	.....	S	D	.....	.....	.....
	.....	.....	.....	.....	S D	D	.....	.....	.....	S D	D	.....	.....	.....
	.....	.....	.....	.....	S D	D	.....	.....	.....	D	D	.....	.....	.....
	.....	.....	.....	.....	S D	D	.....	.....	.....	D	D	.....	.....	.....
	.....	.....	.....	.....	D D	D	.....	.....	.....	.....	D	.....	.....	.....
560	.....	.....	.....	.....	D	.....	.....	.....	.....	.....	.....	.....	.....	.....
	.....	.....	.....	.....	D	.....	.....	.....	.....	.....	.....	.....	.....	.....
	.....	.....	.....	.....	D	.....	.....	.....	.....	.....	.....	.....	.....	.....
	.....	.....	.....	.....	D	.....	.....	.....	.....	.....	.....	.....	.....	.....
	.....	.....	.....	.....	D	.....	.....	.....	.....	.....	.....	.....	.....	.....

TABLE VII.—*Arsphenamine.*

Preparation.	Arsenic content.	Approximate age of sample.	Minimal lethal dose.		Minimal effective dose.		Ratio: $\frac{\text{m. l. d.}}{\text{m. e. d.}}$
			C. c. 1/100 As equiv. solution.	Mgm. per kilo.	C. c. 1/100 As equiv. solution.	Mgm. per kilo.	
Brand A:		*Months.*					
Lot 1	31.86	4	76.6	180	3.0	7.05	25.5
Lot 2	31.86	4	85.1	200	4.5	10.58	18.9
Lot 3	31.67	4	59.1	140	3.0	7.11	19.7
Brand B:							
Lot 1	29.42	4	109.8	280	4.5	11.48	24.4
Lot 2	31.39	4	117.2	280	4.5	10.76	26.0
Lot 3	31.3	1	116.6	280	4.5	10.8	25.9
Brand C:							
Lot 1	30.83	1	98.8	240	4.5	10.94	21.9
Lot 2	31.3	1	100.0	240	4.5	10.8	22.2
Lot 3	31.01	1	82.6	200	4.5	10.89	18.4
Brand D:							
Lot 1	30.73	1	73.8	180	3.0	7.32	24.6
Lot 2	30.78	1	65.6	160	4.5	10.98	14.6
Brand E:							
Lot 1	31.86	3	68.1	160	4.5	10.58	15.1
Lot 2	31.86	3	59.6	140	4.5	10.58	13.2

TABLE VIII.—*Neoarsphenamine.*

Preparation.	Arsenic content.	Approximate age of sample.	Minimal lethal dose.		Minimal effective dose.		Ratio: $\frac{\text{m. l. d.}}{\text{m. e. d.}}$
			C. c. 1/100 As equiv. solution.	Mgm. per kilo.	C. c. 1/100 As equiv. solution.	Mgm. per kilo.	
Brand A:		*Months.*					
Lot 1	18.13	2	115.9	480	2.25	9.32	51.5
Lot 2	18.38	2	98.04	400	3.5	14.28	28.0
Lot 3	18.43	2	103.19	420	3.5	14.25	29.5
Lot 4	18.37	3	78.43	320	3.5	14.28	22.4
Brand B:							
Lot 1	18.79	12	120.32	480	5.0	19.95	24.1
Lot 2	18.88	3	130.98	520	7.5	29.78	17.5
Lot 3	18.69	2	109.73	440	7.5	30.08	14.6
Lot 4	18.32	2	107.58	440	5.0	20.45	21.5
Brand C:							
Lot 1	20.29	3	108.1	400	3.5	12.95	30.9
Lot 2	18.88	1	130.98	520	5.0	19.85	26.2
Lot 3	19.35	1	134.02	520	7.5	29.12	17.9
Lot 4	20.01	3	128.0	480	7.5	28.13	17.1
Brand D:							
Lot 1	20.33	1	97.5	360	5.0	18.45	19.5
Lot 2	20.38	1	97.8	360	5.0	18.4	19.6
Brand E:							
Lot 1	19.31	18	.....	.....	7.5	29.48	.....